The role of pasture in the diet of ruminant livestock

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1 Introduction

The feeding requirements of livestock for macro- (water, energy and protein) and micronutrients (minerals and vitamins) are the bedrock of any sustainable farming enterprise. For monogastric livestock such as chickens (broilers and layers) and pigs, their requirements are defined precisely and delivered in well-balanced rations, whereas for ruminants although requirements are known (Table 1), rationing to match requirements is often not as precise due to several factors as follows: i) intake and quality of pasture at grazing are often not known; ii) variability of the forage component of the diet (mainly silage or hay) at housing can be large across cuts and even within cuts, for example, across a silage clamp face; iii) genetic variability of ruminant livestock (within and across breeds) is wide compared with monogastric livestock making rationing less precise; iv) environment variability (housing and at grazing) is wide compared with much more controlled conditions traditionally when feeding monogastric livestock; and v) the foregut microbial fermentation and the symbiotic role rumen microorganisms play in metabolism and nutrient composition post-rumen. The complexities around these factors lead many ruminant livestock enterprises to optimise rations in order to meet production targets (milk yields and live weight gain), through supplementation of the diet with human-edible

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Table 1 Macro- and micronutrient requirements of typical ruminant livestock

	Dairy[1]		Beef[2]		Sheep[3]	
	Daily requirement	Nutrient density	Daily requirement	Nutrient density	Daily requirement	Nutrient density
Water	60–114 L	–	27–55 L	–	3.6–5.2 L	–
Energy	230 MJ/d	12 MJ/kg DM	73–88 MJ/d	11 MJ/kg DM	13–16 MJ/d	10 MJ/kg DM
Protein	3.08 kg CP/d	160 g CP/kg DM	0.9 kg CP/d	130 g CP/kg DM	0.2 kg CP/d	120 g CP/kgDM
Ca	80 g/d	4 g Ca/kg DM	36 g/d	5 g Ca/kg DM	6.6 g/d	4 g Ca/kg DM
P	25–50 g/d	1–2 g P/kg DM	18 g/d	2.5 g P/kg DM	3.2 g/d	2 g P/kg DM
Mg	40 g/d	2 g Mg/kg DM	14 g/d	2 g/kg DM	3.0 g/d	1.8 g/kg DM
Na	30 g/d	1.5 g Na/kg DM	7 g/d	1 g/kg DM	3.0 g/d	1.8 g/kg DM
K	80 g/d	4 g K/kg DM	49 g/d	7 g/kg DM	12.8 g/d	8 g/kg DM
S	30 g/d	1.5 g S/kg DM	10.5 g/d	1.5 g/kg DM	4.2 g/d	2.6 g/kg DM
Co	2 mg/d	0.1 mg Co/kg DM	0.7 mg/d	0.1 mg/kg DM	0.3 mg/d	0.2 mg/kg DM
Cu	200 mg/d	10 mg Cu/kg DM	70 mg/d	10 mg/kg DM	17.6 mg/d	11 mg/kg DM
I	10 mg/d	0.2 mg I/kg DM	3.5 mg/d	0.5 mg/kg DM	1.3 mg/d	0.8 mg/kg DM
Se	3 mg/d	0.3 mg Se/kg DM	0.7 mg/d	0.1 mg/kg DM	0.3 mg/d	0.2 mg/kg DM
Zn	600 mg/d	30 mg Zn/kg DM	210 mg/d	30 mg/kg DM	53 mg/d	33 mg/kg DM
Mn	500 mg/d	25 mg Mn/kg DM	280 mg/d	40 mg/kg DM	64 mg/d	40 mg/kg DM
Vitamin A	50 000 IU/d	2500 IU/kg DM	30 000 IU/d	4000 IU/kg DM	360 IU/d	226 IU/kg DM
Vitamin E	1000 IU/d	90 IU/kg DM	1250 IU/d	170 IU/kg DM	5 IU/d	3.1 IU/kg DM

[1] Values relate to the typical requirements and dry matter intake for a dairy cow weighing 650 kg and producing on average 30 L of milk per day.
[2] Values relate to the typical requirements and dry matter intake for a growing beef animal 300–400 kg live weight gaining 1 kg/day.
[3] Values relate to the typical requirements and dry matter intake of a finishing lamb 25–40 kg live weight.

concentrate feeds (cereals, grains and pulses) or/and rumen-protected products. In a recent review on the use of human-edible feed by ruminant livestock, Wilkinson and Lee (2017) concluded that whilst human-edible feeds have vital roles to play in complementing grazed pasture and conserved forages, to increase total diet dry matter (DM) intake and rectify nutritional imbalances, (especially for high-yielding dairy cows), grazed pasture and forage should be the single most important feed for ruminants. This is due to its low unit cost, widespread global availability, rumen and animal health benefits (ruminants evolved to utilise fibre, high concentrate diets can lead to ruminal acidosis) and reduced competition for human-edible feed. The use of pasture and by-products underlies the ability to sustainably deliver future ruminant livestock production systems and their future role in food security (Eisler et al., 2014; Smith et al., 2016).

Livestock breeding has significantly improved the performance of animals over the last 30 yrs (Dumont et al., 2014) with consequent increases in nutrient demands, which can outstrip those provided in forage (Table 2). Equally, as livestock breeding goals have concentrated on performance traits, forage breeding goals also need to be delivered to match the nutrient demands of these new elite animals. Breeding forage grasses has provided a means of improving the efficiency of milk and meat production at the same time as reducing environmental pollution at a minimal cost to farmers (Abberton and Marshall, 2005). Genetic gains in early spring growth and annual DM yield have been a major focus. However, improving the nutritive value of forage (e.g. water-soluble carbohydrate; WSC) has also played a significant role through maximising microbial capture of nutrients in

Table 2 Nutritional value and variability of typical temperate forage grass

	Typical range	
Water (%)	60	83
Energy (MJ/kg DM)	9	12
Protein (g/kg DM)	70	330
Ca (g/kg DM)	2	10
P (g/kg DM)	2	5
Mg (g/kg DM)	1	4
Na (g/kg DM)	0.5	10
K (g/kg DM)	20	24
S (g/kg DM)	0.5	2
Co (mg/kg DM)	Tr	0.1
Cu (mg/kg DM)	5.0	7.0
I (mg/kg DM)	0.05	0.5
Se (mg/kg DM)	0.03	0.15
Zn (mg/kg DM)	20	24
Mn (mg/kg DM)	40	80
Vitamin A (IU/kg DM)	15 000	200 000
Vitamin E (IU/kg DM)	233	368

the rumen and improving efficiency and product quality (Merry et al., 2006). Novel plant traits, such as polyphenol oxidase (PPO) in red clover, which has been shown to improve nitrogen use efficiency (NUE) and protect polyunsaturated fatty acids (PUFA) through the rumen (Lee, 2014), are additional traits which could be incorporated into forage breeding programmes. Breeding objectives should target ruminant nutrition demands (Table 1; macro- and micronutrients) so that the genetics of the plant is developed to mirror the requirements of rumen microbial fermentation and subsequently the host's genetics to deliver more sustainable ruminant livestock production systems driven from pasture.

2 Energy

Energy from pasture for ruminants comes in three main forms: i) WSC – readily available energy; ii) fibre – slow release energy via the fermentation in the rumen; iii) lipid – non-fermentable energy.

2.1 Water-soluble carbohydrates

WSC (glucose, fructose, sucrose and fructans) and starch are used by plants as the substrate for growth and respiration. Adequate reserves are important in perennial plants for winter survival, early spring growth initiation and regrowth initiation after herbage removal due to a shortage of photosynthetic products related to plant demand (White, 1973). WSC also plays a protective role in grasses such as perennial ryegrass (*Lolium perenne* L.) through their accumulation during cold acclimation, helping to lower cellular freezing point and improve cellular stability at low temperatures (Hoffman et al., 2010). WSC content of species such as ryegrass (*Lolium* spp.) varies with types (ecotypes vs cultivars), harvest date, ploidy level (diploid vs tetraploid; McGrath et al., 2014), defoliation regime (Turner et al., 2006a,b), sward type (da Silva et al., 2014), nitrogen (N) fertilisation rate (McGrath et al., 1992), time of the day, season (Taweel et al., 2005) and weather conditions (Hoekstra et al., 2007), among others. These factors result in the WSC content of ryegrass varying considerably from 5 to 40% of DM (McGrath, 1988).

WSC in pasture also plays a vital role in ruminant nutrition, and is particularly associated with NUE. The microbial protein synthesis in the rumen provides the bulk of amino acids for digestion and metabolism by the animal, with typical feeds resulting in around 150–200 g of microbial protein formed per kg of organic matter digested in the rumen. When supplies of readily available energy (mainly WSC from pasture) in the rumen are sufficiently high, rumen microbes incorporate N (ammonia, amino acids and peptides) released from the rapid breakdown of forage protein and non-protein N sources to form microbial protein, thereby improving the efficiency of N capture from the diet into product (meat and milk). However, when WSC is not adequately available to balance the rapid release of soluble N (ammonia) in the rumen, the microbes rely on slowly releasing sources of energy such as fibre or metabolism of amino acids. This results in ammonia buildup, due to low incorporation into microbial protein, and loss through the rumen wall, decreasing the efficiency of microbial protein synthesis (Lee et al., 2003) and total NUE of the diet (Merry et al., 2006; Fig. 1). WSC from forage has also been shown to increase the glucogenic:lipogenic volatile fatty acid (VFA) ratio (Lee et al., 2003), increase the rate of fermentation and reduce methane formation *in vitro* (Purcell et al., 2014), all of which will

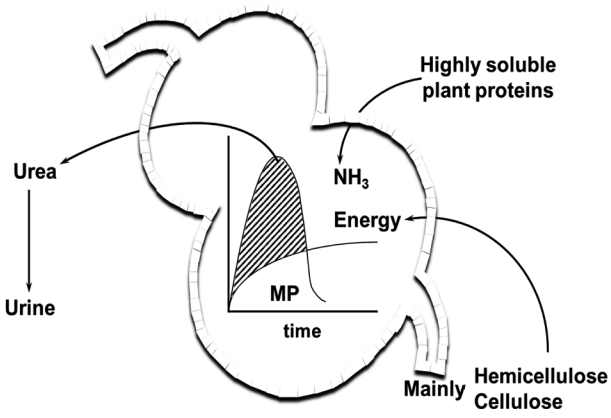

Figure 1 Nitrogen and energy balance in the rumen. The graph represents the metabolism of plant protein (top curve) and plant energy (bottom curve) in the rumen, where they are balanced, microbial protein (MP) is formed, the more rapid metabolism of plant protein to NH_3 (hatched area) results in loss out of the rumen and conversion to urea.

improve energy utilisation via a greater supply of glucose, greater DM intake and lower gross energy loss, respectively. These findings indicate a potential for improved animal productivity on 'high-sugar' cultivars (WSC concentration of circa 20–40% of DM) through an increase in the efficiency of N and energy utilisation. This has led to a great interest in developing grass cultivars, particularly ryegrass, with elevated levels of WSC in the leaves over the last 50 yrs (Parsons et al., 2011).

In vivo, Fraser et al. (2015) showed that increases in WSC (226 vs 118 g WSC/kg DM) that were accompanied by a reduction in neutral detergent fibre (NDF) in grazed pasture increased voluntary DM intake in weaned lambs, as well as lowering methane emitted (16.4 vs 17.7 g/kg DM intake) and the proportion of gross energy intake excreted as methane (0·052 vs 0·056 MJ/MJ). When increased WSC content was associated with lower crude protein (CP) in the forage, a lower urinary N excretion and milk urea concentration, and greater faecal N excretion and milk N secretion in dairy cows was reported (Staerfl et al., 2012; Ellis et al., 2011). In sheep fed with cultivars containing high WSC levels compared to a control grass, greater carrying capacity, average daily gain (Lee et al., 2001; Cosgrove et al., 2015) and reduced urinary N excretion relative to N intake (Jonker et al., 2015) have been reported. Similarly, steers fed with perennial ryegrass containing greater levels of WSC than a control cultivar (243 vs 161 g/kg DM) showed increased DM intake (9.3 vs 6.7 kg/d) and flows of non-ammonia N to the duodenum, as well as absorption of amino acids from the small intestine while decreasing rumen ammonia levels and increasing the glucogenic:lipogenic VFA ratio (Lee et al., 2002). Similar results were obtained in dairy cows, where an increase in the WSC concentration of the diet increased grass herbage DM intake, digestible DM intake and efficiency of utilisation of dietary N, thereby increasing milk yield and protein content, and reducing N excretion in urine and urea N in milk (Staerfl et al., 2012; Miller et al., 2001; Moorby et al., 2006). These studies suggest that the benefits of high WSC levels in pasture for increasing NUE of milk are more likely to occur when the WSC:CP ratio is above 0.75 (Pacheco et al., 2009). Figure 2 shows an overall relationship between WSC content of forage and NUE indicators, with a reduced

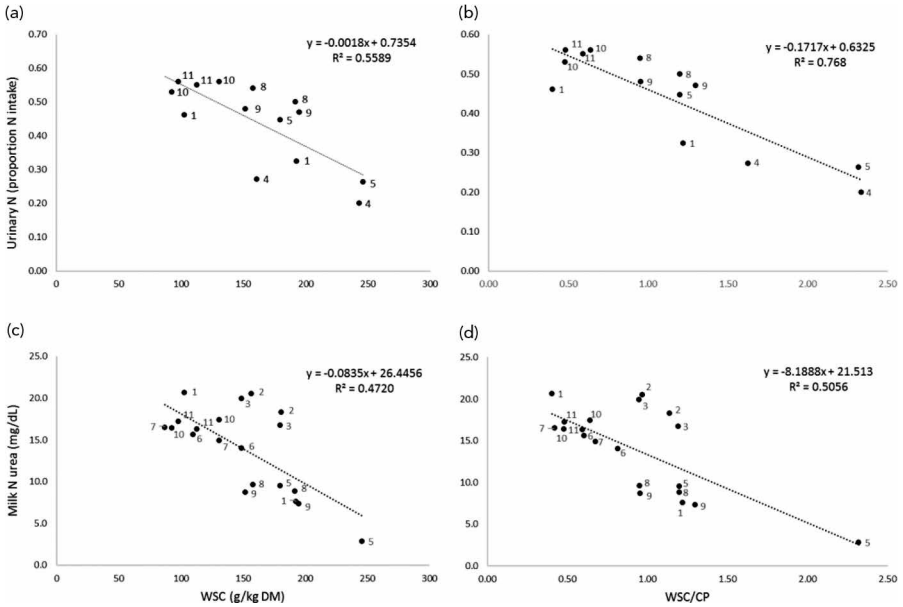

Figure 2 Relationship between WSC content of forage and (a) urinary N excretion, as a proportion of N intake, (b) milk urea N content, and between WSC to CP and (c) urinary N excretion and (d) milk urea N content. Each point represents a single experiment, or a particular Latin square (LS) or year within an experiment. 1: Staerfl et al. (2012); 2: Taweel et al. (2005) LS1; 3: Taweel et al. (2005) LS2; 4: Moorby et al. (2006); 5: Peyraud et al. (1997); 6: Tas et al. (2006a) 2002; 7: Tas et al. (2006a) 2003; 8: Tas et al. (2006b) LS1 2000; 9: Tas et al. (2006b) LS2 2000; 10: Tas et al. (2006b) LS1 2001; 11: Tas et al. (2006b) LS2 2001.

excretion via milk and urine as WSC increases. Moreover, this association is stronger when considering the WSC-to-CP ratio instead of only WSC content, in agreement with previous studies (Staerfl et al., 2012; Ellis et al., 2011; Jonker et al., 2015).

WSC content is also vital for silage fermentation, with its rapid conversion to lactic acid by lactic acid bacteria (LAB) ensuring a rapid drop in pH, reduced proteolysis and a stable fermentation (Davies et al., 1998). When herbage has low concentrations of WSC for silage formation, LAB may follow a more heterofermentative pathway (multi-fermentation pathways and end products) instead of a homofermentative pathway, which can result in a decrease in silage quality (more acetic and less lactic acid), a reduction in intact ribulose-1,5-bisphosphate carboxylase (RUBISCO) and an increase in non-protein N (Huhtanen et al., 1998). At feedout, silage DM intake is also positively correlated with the concentration of residual WSC, promoting greater milk fat concentrations and yields in mid-lactation Holstein-Friesian dairy cows, with lower excretion of feed N into urine (Huhtanen et al., 1998; Moorby et al., 2006). It has also been reported that high-WSC silage resulted in a greater NUE (0.86 vs 0.65 g of microbial N/g of feed N) and a lower rumen ammonia-N concentration (99.4 vs 127.4 mg/L) than for a control grass silage (Merry et al., 2006), probably due to a combination of lower DM intake on the control grass silage and the extra readily available energy in the high-WSC grass silage (85.7 vs 30.1 g/kg DM).

Feeding high WSC forages has also been shown to influence the flavour of milk by reducing the formation of amino acid degradation products, including skatole and indole, through a greater supply of energy-reducing amino acid catabolism in the rumen (Tavendale et al., 2006). Furthermore, a reduction in the N emission potential of the manure may also provide an environmental benefit (Staerfl et al., 2012).

2.2 Fibre

The bulk of the energy in herbage is present as fibre derived from cell walls. The digestibility of these polysaccharides in the rumen is a key determinant of livestock productivity. The cell walls consist mainly of cellulose, hemicellulose and lignin and minor components such as pectin and protein. In feed rations, these fractions are represented simply by their digestibility in different detergent solvents, namely acid detergent fibre (ADF) which includes cellulose and lignin, and NDF which includes hemicellulose, cellulose and lignin. These simple nomenclatures devised by Peter Van Soest in the 1960s have been used by nutritionists ever since to represent this complex group of polysaccharides. Fibre is vital for deriving energy and maintaining a healthy rumen through: i) providing substrate for fibrolytic fermentation by rumen microorganisms, resulting in the formation of the main energy substrate for ruminants, VFA (acetate, butyrate and propionate), which feed into Kreb's citric acid cycle (acetate and butyrate) or glycolysis (propionate) for the formation of adenosine triphosphate; ii) stimulating the production of saliva through the action of chewing and rumination of physically effective fibre; this saliva acts as a buffer in the rumen, controlling pH homeostasis and helping to maintain a healthy rumen environment. Therefore, too little fibre is a problem but likewise too much fibre is also problematic as it will reduce DM intake, and, subsequently, energy intake and performance. Based on this fibre balance, a good ruminant ration should contain at least 40% forage of which 75% of the NDF should be from the forage element with total NDF not below 30% of total DM.

The macromolecules' cellulose and hemicellulose are in principle easy to degrade by rumen microbes but the presence of lignin limits the accessibility of the carbohydrates, thereby reducing degradability. In the cell walls of forage grasses, ferulic acid is esterified to arabinoxylans and participates with lignin monomers in oxidative coupling pathways to generate ferulate–polysaccharide–lignin complexes that cross-link the cell wall (Buanafina, 2009) and reduce degradability in the rumen. Lignin is a polymer of phenol components which represents between 2 and 10% of the cell wall depending on the origin of the plant, maturity, etc. (grass contains 30–65 g lignin/kg NDF). Only certain species of aerobic white rot fungi have the capability of degrading lignin which is not present in the rumen (anaerobic). Cellulose, the major component of plant cell walls, is a polymer consisting of a linear chain of several hundred to tens of thousands of β (1→4) linked D-glucose units. The macromolecules can associate with microfibrils to strengthen and stabilise the cell wall. The amount of hemicellulose in the plant cell wall is about 50% that of cellulose, but is highly dependent on plant species, physiological stage and environmental conditions. Contrary to cellulose, hemicellulose is a polymer of many different sugars (glucose, xylose, mannose, fucose, galactose, rhamnose, arabinose and apiose), is branched and has an amorphous structure.

The young plant cell wall consists mainly of the primary wall and cells are connected to each other by middle lamellas. The young cell walls do not contain lignin and are highly degradable in the rumen. Upon ageing and maturation, the secondary cell wall develops, making cellulose and hemicellulose inaccessible to enzymes and microorganisms, as

explained above. The degradability of plant cell walls can be influenced by many factors, such as chemical composition, genotype, tissue composition, physical properties, maturation, harvest date and growing conditions. The relationship between lignin content and cell wall degradability has been proven many times. Buxton and Russell (1988) showed that the relationship between lignin and degradability for grass has an r^2 of 0.93 and for legume stems, the value is 0.84. Although the relationship is rather strong, it cannot be said that only lignin content determines cell wall degradability. For instance, Cone and Engels (1993) showed that the cell wall degradability of a brown midrib mutant and a normal maize (*Zea mays*) cultivar was the same at the end of the growing season, although the brown midrib mutant contained about half the lignin of the normal cultivar. Using an advanced technique, namely pyrolysis mass spectrometry, He et al. (2017) recently showed that the composition of lignin plays a more decisive role in cell wall degradability than the lignin content (see also Jung et al. (1993)). The chemical composition of forage determines the degradability of plant cell walls in the rumen, subsequently influencing rumen emptying, which is important for DM intake. Reducing the level of ferulate–polysaccharide–lignin complexes that cross-link the cell wall has long been a target to enhance the digestibility in grasses. Some natural variation has been exploited, for example, low xylan ferulate maize fodder gave improved milk yields when fed to cattle (Jung et al., 2011). This could be pursued in pasture species such as ryegrass to increase the digestibility and subsequent DM intake and energy supply from forage grasses.

Not all tissues in a plant have the same content of lignin. Lignin is mainly situated in the sclerenchyma around the vascular bundles and near the epidermis. Other tissues, such as the parenchyma, contain much less lignin and the phloem tissue contains no lignin. This means that the distribution of the tissues has an influence on the lignin content in the plant. Some cultivars can have more vascular bundles than others and the sub-epidermal layer of sclerenchyma can vary between cultivars. This is one of the reasons why the genetic background of the forage plays a role in the degradability of the cell walls. Of course, genetics determines much more in the plant than only the tissue distribution; lignin content and lignin composition are also genetically determined. Among maize cultivars, the NDF digestibility can vary between 40 and 60%. Also within a plant, there can be a large difference in chemical composition and degradability. For instance, the lower parts of plants are older, with much higher lignin content than the upper parts. Maturation has a big influence on the chemical composition of the cell walls and subsequently on degradability in the rumen. Harvesting the forage at the right growth stage is an easy way to obtain highly digestible forage. However, the yield of a young crop is always much lower than that of a more mature crop. The challenge for the farmer is to harvest a crop with an optimal digestibility, but also at an optimal yield.

2.3 Lipids

Lipids are the most energy-dense dietary nutrients (38 kJ/g); they are not fermented in the rumen and so are directly available to the ruminant. As energy supply to ruminants on pasture systems often limits production, a higher concentration of lipid in pasture grasses could significantly increase the production for the same DM input. For example, an increase of 3% lipid in grasses could supply an extra 1.1 MJ of gross energy per kg DM, significantly increasing animal performance (e.g. for dairy cows, 5 MJ metabolisable energy equates to approximately 1 L of milk). Typically, forage lipid only makes up 3–5% of DM, with the majority present in photosynthetic membranes of leaves. The metabolisable

energy content of livestock diets can be increased by supplementing lipids with oils up to an optimum of 6% of DM, as greater levels have been shown to significantly reduce fibre degradability in the rumen (McAllan et al., 1983). Oil supplementation not only has been shown to boost productivity and product quality (fatty acid composition) but may also reduce enteric methane emissions (Beauchemin et al., 2007). Approximately 70% of the UK methane emissions are a result of agricultural activities and 85% of those are derived from livestock. While there are well-known dietary manipulations that can be used to reduce methane emissions from ruminant livestock (e.g. by increasing starch and free oils in the animal's diet; Beauchemin et al., 2008), achieving this at grazing, without relatively expensive and labour-intensive supplementation regimes, is not easy. Therefore, to reduce methane excretion at grazing, strategies for improving the grazed material must be adopted. It is calculated (Beauchemin et al., 2008) that methane emissions from ruminants (in g/kg of DM consumed) could be reduced by 5.6% for each 1% increase in supplemental lipid. Increasing lipids in grasses will also significantly improve the supply of omega-3 PUFA, which has beneficial effects in terms of animal health and fertility (Richardson et al., 2013). There are also significant concerns about the potentially negative effects of meat and milk fat consumption on human health (Garnett, 2009). However, forage-fed animals produce meat and milk with a lipid composition that is more favourable to human health than that derived from concentrate-fed animals (Daley et al., 2010). This is particularly associated with a more 'beneficial' ratio of omega-3:omega-6 PUFA as forages have a high omega-3 PUFA content with total lipid typically comprising 50–75% omega-3 and 6–20% omega-6 (Dewhurst et al., 2003). Pasture species with a fatty acid content that further enhances the beneficial impact of forage feeding would be an important tool in the development of a sustainable livestock sector, meeting the needs of the value chain and consumers.

Much is known about the genetics of lipids and fatty acid content in crops such as rapeseed (*Brassica napus;* Ecke et al., 1995; Barker et al., 2007), linseed (*Linum usitatissimum;* Ecke et al., 1995; Cloutier et al., 2011) and soybean (*Glycine max;* Csanadi et al., 2001; Hyten et al., 2004). The oil seed lipids are particularly rich in triacylglycerides (TAG). In contrast, the lipids in the leaves of forage crops are derived chiefly from the cell and organelle membranes, particularly within the chloroplasts. Their main constituents are phospholipids (mainly phosphatidylcholine) and galactosylglycerides, with the former found more commonly in the cell and organelle membranes, while the latter are the main components of the chloroplast thylakoid. The fatty acid concentration of a perennial-ryegrass-mapping family, produced at the Institute of Biological, Environmental and Rural Science in Aberystwyth in the UK, showed a range from 1 to 5% of DM, and the estimates of broad sense heritability of the main constituents ranged between 0.4 and 0.8 (Hegarty et al., 2013). These numbers are highly encouraging when considering the scope for genetic improvement in this species. Hegarty et al. (2013) identified reliable Quantitative Trait Loci (QTLs) for 18:3 omega-3, 18:2 omega-6 and 18:0 in this population, and potential candidate genes underlying some of these QTLs providing a foundation for further dissection of this trait. Alternatively, approaches could be developed to increase the TAG proportion of forage lipids as with oil seeds. TAG represents much less than 0.1% of DM; however, several mutations that stimulate TAG synthesis and repress breakdown have recently been discovered, and these can substantially enhance the content in the leaves of the model plant *Arabidopsis thaliana* (Pant et al., 2015). This increase in the fatty acid content is much larger than any so far achieved by conventional breeding and could be a basis for forage improvement programmes. However, concerns regarding the impact

on fibre digestion in the rumen with fatty acid supply greater than 6% of DM have first to be alleviated. Recent research at Rothamsted Research in the UK (P. Eastmond, H. Van-Erp, B. Van Selm, C. Hodgson and M.R.F. Lee, unpublished) has compared *Arabidopsis* mutants with a total lipid content ranging between 7.2 and 8.1% of DM against wild-type material (3.1–4.0% lipid of DM) when incubated for 12 h in rumen fluid *in vitro* for the impact on DM and fibre digestibility. A third treatment was also included of the wild type + free oil to raise the total level of lipid to that of the mutant in the incubation (ca. 8% of DM). Although there was no difference in fibre digestibility across the treatments, total NDF was lower in the mutant than the wild type (109 ± 25.2 vs 167 ± 16.9 g NDF/kg DM). However, total DM disappearance during the 12-h incubations was significantly reduced for the wild type + free oil than the other treatments, with the mutant and wild type not different from each other. This suggests a lower impact on DM digestibility when the lipid was cellular bound rather than free oil, indicating the potential for developing forages which express higher lipid content without reducing digestibility in the rumen (Fig. 3).

3 Proteins

The main proteins in the forage leaf tissue are RUBISCO and the chloroplast lamellar light harvesting proteins associated with photosystems I and II (Cashmore, 1976). However, this is often over simplified by animal nutritionists, as is also the case with fibre (see the section on NDF and ADF), by referring to CP content of a diet. This assumes protein content based on the mean N content of amino acids (16%) and therefore predicts by multiplying the N content, which is much easier to measure, by 6.25. The protein adequacy in any feed can be divided into the quantity and quality of the protein. The quantity of protein in grass varies greatly (Table 2), depending on the sward type, growth stage, fertiliser regime and time of the year. The quality of protein is defined by the amino acid composition, and the proportion which is rumen-degradable (RDP) and thus available to the microbes to assimilate microbial protein (see the section on WSC) and the fraction which is undegraded in the rumen (Undegraded Dietary Protein; UDP), which although unavailable for the

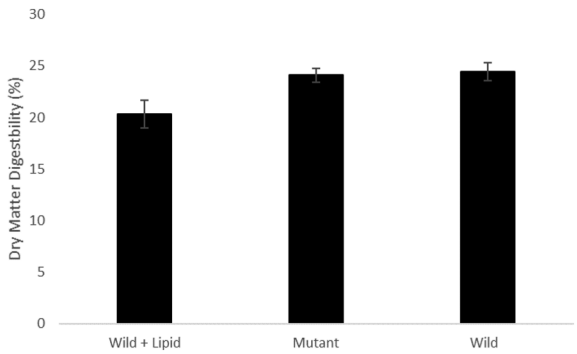

Figure 3 Dry matter disappearance during 12-h incubation in rumen fluid and buffer from Arabidopsis mutants with high cellular lipid (7.2–8.1%), wild-type Arabidopsis and wild type + free oil to bring total lipid to the level of the mutant (Unpublished data).

assimilation of microbial protein may be available to the animal in the small intestine. Plant-based proteins are typically low in the amino acids methionine, lysine and histidine. Whilst there are no essential amino acids for ruminants, due to the ability of the rumen microbes to synthesise all 22 (including pyrrolysine and selenocysteine) amino acids from carbohydrate C-skeletons, sulphur, selenium and non-protein nitrogen sources, high-performing animals such as high-genetic merit Holstein dairy cows producing 40+ L of milk per day can have limiting amino acids for production. For total mixed rations based on maize silage and soybean (common dairy ration), the most limiting amino acids are often methionine and lysine, which can then be supplemented in the form of rumen bypass amino acids to ensure that they are not metabolised in the rumen (Broderick et al., 2009). For high-forage (grass) diets, the first limiting amino acid is usually histidine due to the low level of histidine in pasture and the greater reliance on microbial protein formation in the rumen from RDP, which is also low in histidine (Lee et al., 2014; Vanhatalo et al., 2009). Protein quality tends not to be an issue for young stock, suckler cows or finishing cattle on grass, but for freshly calved cows in springtime, there is a need for some quality protein through the supplementation for the first 6 weeks of lactation. Also, while autumn grass has adequate protein for late-lactation spring calving cows, freshly calved autumn calving cows may need some quality protein in the ration to meet their requirements (Tables 1 and 2).

The concentration of CP in grass pre-grazing typically contains more than 200 g/kg DM (Holmes et al., 2002; Wilkinson et al., 2014), excessively high in relation to animal requirements (Table 1). Although there is a marked decline in CP from about 330 g CP/kg DM at the three-leaf stage of growth to about 70 g CP/kg DM at full flowering (Beever et al., 2000), the problem of excess N intake (and excretion) is compounded by grazing selection. Selection of leaf in preference to stem results in the grazing animal consuming herbage of higher quality than the average for the whole sward. This grazing selection differential (the difference between what an animal consumes and the mean of cut samples) for CP has been quantified at between 1.1 and 1.5, depending on the efficiency of pasture utilisation (Stockdale and Dellow, 1995; Jacobs et al., 1999). Thus, at a relatively high efficiency of pasture utilisation (e.g. 75%), which would be a reasonable target under well-managed grazing systems, the grazing animal can consume herbage about 10% higher in CP concentration than the average, that is, 220 g CP/kg DM in the herbage DM intake when the average for the pre-grazed pasture allowance is 200 g CP/kg DM. With more mature herbage on offer and/or higher quantities of residual herbage (and lower efficiency of utilisation), the grazing animal effectively negates any reduction in overall pasture CP concentration by rejecting stem and mature leaf of below-average CP.

Forage breeding has improved the balance of readily available energy and RDP. Grasses with higher levels of WSC, as already mentioned, have been used to increase the supply of readily available energy to increase NUE (Lee et al., 2003), milk yield (Miller et al., 2001) and animal growth rate (Lee et al., 2001). Other improvements centre around either reduction in total protein supply or reduction in the availability of forage N in the rumen. In a review by Abberton and Marshall (2005), they indicate the importance of breeding goals for forage legumes to reduce the contribution of clovers to both direct (leaching) and indirect (through animal returns) N and phosphorus pollution. This would predicate the breeding of lower N-containing lines of forage legumes such as white clover (*Trifolium repens*) for grazing systems, and the enhancement of traits affecting protein breakdown in the rumen and silo. One such target has been identified as PPO in red clover which has been shown to improve NUE through reducing the digestibility of plant protein in the rumen and increasing the proportion of dietary protein flow out of the rumen (Lee, 2014).

4 Minerals and vitamins

Often the micronutrient (minerals) concentrations of forages are not high enough to meet the nutritional needs of livestock (Tables 1 and 2). Consumption of low-mineral density diets in livestock results in suboptimal growth, limited production and an array of health problems. Prevention and early detection of diseases are critical for the development of sustainable livestock systems. Low mineral bioavailability from pasture causes many grazing livestock diseases, for example, low serum Ca levels (hypocalcaemia or milk fever), low plasma Mg levels (hypomagnesaemia or grass tetany), Cu deficiency (swayback), Se and vitamin E deficiency (white muscle disease) and Co deficiency (pine), further summarised in Table 3. The interaction of minerals, either in the soil, reducing uptake by plants (e.g. high Ca in chalky soils causes poor availability of Mg; application of K fertilisers inhibits the uptake of Mg in grass), or in the rumen, reducing absorption (e.g. formation of thiomolybdate reducing Cu absorption), further complicates the delivery of minerals to livestock. It is not just a question of what minerals are present, but their level of bioavailability that is critical, for example, apparent availabilities of 0.3–0.4 are commonly reported for Ca, P and Mg in herbage. Greene (2016) assessed the mineral supplementation needs in pasture-based beef operations in the Southeastern United States. The paper concluded that Ca, P, Mg, Na, Cl, Zn, Cu, I, Mn, Se and Co required supplementation, although the amounts to be supplemented differed based on multiple interactions occurring within the soil and the rumen, and the requirement driven by the physiological state of the animal. Hypocalcaemia in early lactation dairy cows is a perfect example of some of these complexities, where a low-Ca diet is required prior to parturition in order to prime homeostatic mechanisms around bone Ca remobilisation which can be difficult due to the relatively high levels of Ca in most pastures. Alternatively, to assist Ca bone remobilisation, a diet should be acidified, which will mean low cations (predominately Na and K) and higher anions (predominately Cl and S). Again, this is difficult to achieve solely from pasture. Such issues are relatively simple to fix through supplemented rationing when the animals are indoors, and such disorders are indeed more prone to high-performing animals, for example, high-yielding dairy cows, which traditionally spend more time indoors. However, grazing animals do suffer from several disorders and the solutions are not as simple to apply.

The absorption of minerals from the soil to pasture is driven by soil properties. For example, Se exists as different chemical forms: elemental Se (Se^0), selenide (Se^{2-}), selenite (SeO_3^{2-}), selenate (SeO_4^{2-}) and organic Se selenomethionine (SeMet) and selenocysteine (SeCys). These chemical forms have different levels of uptake by forage, with selenate being more easily adsorbed compared to selenite under natural soil conditions (Sors et al., 2005). The soil chemical environment determines the proportion of Se forms, with alkaline conditions favouring selenite (Missana et al., 2009). A soil redox potential (Eh) above 500 favours selenate and selenous acid (H_2SeO_3) formation, whereas −500 Eh favours hydrogen selenide (H_2Se) formation (Fig. 4; Mikkelsen et al., 1989). Archer (1971) carried out one of the few reviews on mineral content of pastures and amendments for the improvement of pasture to supply minerals to grazing ruminants. The report concluded that although mineral content of the soil is of primary importance, plant species, age of plants present, drainage conditions, lime and fertiliser treatments also exert an influence. The consideration of these factors was necessary on a case-by-case basis to effectively deliver the key minerals to livestock solely from pasture. Factors such as drainage, lime and fertilisation are driven by soil properties as exemplified by Se, with certain minerals

Table 3 The roles and deficiency symptoms of micronutrients in livestock

Micronutrient	Role in livestock	Symptoms of deficiency comments
Zn	Enzyme activation and function.	Stiff, swollen joints, reduces immune responses and disease resistance. Response to Zn supplementation is rapid, excess Zn intake can lead to Cu deficiency.
Fe	Enzyme and protein function, formation of blood haemoglobin.	Anaemia, reduced feed intake and weight gain. High dietary consumption of Fe reduces Cu status in ruminants.
Mn	Enzyme activation and function.	Skeletal abnormalities, retarded growth, and reproductive failure. Requirements by ruminants are low, so deficiency is rare.
Cu	Haemoglobin formation, pigments and enzyme function.	Anaemia, bone disorders, decolouration of hair, poor growth, digestive upsets, brain and spinal cord lesions and infertility. In sheep deficiency for the pregnant ewe can result in 'swayback' disease of offspring.
Se	Main cellular anti-oxidant through role in glutathione peroxidase, also essential in thyroid function.	White muscle disease, poor growth, infertility. Deficiencies in Se reduce the ability of cows to kill bacteria in milk, enhances immune response.
Ca	Structural components in bones and teeth, blood clotting, cardiac regulation.	Rickets, retarded growth and development. Legumes are generally higher in Ca than grasses, cereals are low in Ca, cattle fed with high grain diets require supplementation.
Mg	Activation of over 300 enzymes, transmission of the genetic code, Anorexia, convulsions, calcification of soft tissue.	Mg deficiency common in lactating cows grazing spring pastures – Hypomagnesaemia.
K	Enzyme reactions, formation of intracellular fluid.	Weight gain and reduced feed Intake. Cereal grains are often deficient in (<0.5%) in K, supplementation may be required.
Co	The main function of cobalt in ruminants is to be a component of vitamin B_{12}, also known as cobalamin. Vitamin B_{12} is an essential cofactor for the function of Methionine synthase and Methylmalonyl–CoA mutase.	Pine - lethargy, reduced appetite, poor-quality wool with an open fleece, small size and poor body condition despite adequate nutrition.
Na	Helps keep the water and electrolyte balance of the body. Sodium is also important in how nerves and muscles work.	Hyponatraemia - symptoms are not usually very specific and can include vomiting, tiredness, muscle spasms and seizures.

(*Continued*)

Table 3 (*Continued*)

Micronutrient	Role in livestock	Symptoms of deficiency comments
I	Metabolic activity through the thyroid hormones.	Lethargy and in extreme cases goitre. Often associated with goitrogens found in certain plants which influence uptake of Iodine.

showing conflicting responses; for example, uptake of Se, Mn, Fe, Cu, Zn, B and Co is favoured in more acidic soils, whereas uptake of P, K and Mo is more favoured in alkali soils. In terms of plant species, clear differences are reported; for example, common heather (*Calluna vulgaris*) and buttercup (*Ranunculus* spp.) show higher levels of Co and Cu, respectively, than grasses; cocksfoot (*Dactylis glomerata*) is higher in Mn and Mo than ryegrasses or red clover when grown as a mixed sward but Co is higher in ryegrass and red clover than cocksfoot (Mitchell, 1951). More recently, Pirhofer-Walzl et al. (2011) showed that the mineral composition of grassland could be considerably improved by the addition of herbs. Such multi-species swards have been shown to elicit high levels of performance, possibly because of the better supply of micronutrients (Grace et al., 2016). Careful attention to the mineral status of soils and pasture can significantly improve the health status of a herd and flock. Improving soil fertility and designing swards to provide vital minerals to grazing livestock provide a great opportunity to improve sustainability

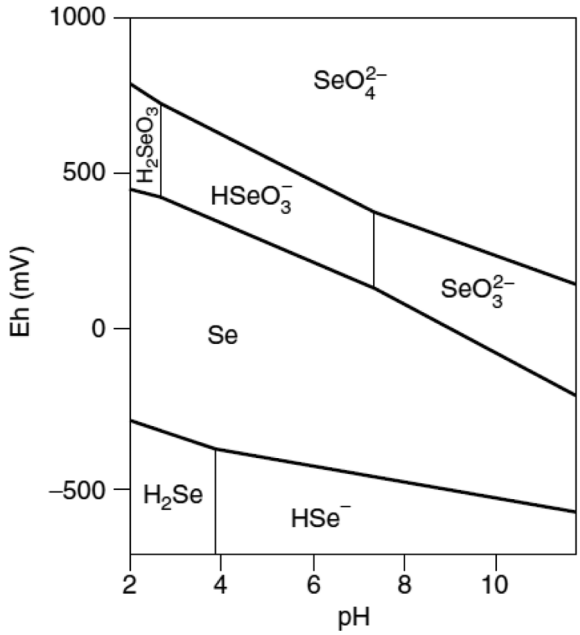

Figure 4 The speciation of selenium as affected by pH and the oxidation–reduction potential Eh, showing the distribution of selenate, selenite and associated acids (Mikkelsen et al., 1989).

through a greater utilisation of pasture, lower supplementation, reduced veterinary fees and improved performance.

Vitamin requirements for ruminants from pasture are predominately associated with vitamins A and E (Tables 1 and 2). Given adequate supply of Co, the rumen microbes can synthesise all the animal's requirements of B vitamins (B1, thiamine; B2 riboflavin; B3, niacin; B5, pantothenic acid; B6, pryridoxine; B7, biotin; B9, folic acid; B12, cobalamins), although certain anti-nutritional factors (ANFs) can reduce the ability to produce thiamine (see the section below). Vitamin K is also synthesised in the rumen and the lower gastrointestinal tract by microbes. Ruminants can synthesise their own requirements for vitamin C (unlike man) and adequate vitamin D is supplied through the conversion of 7-dehydrocholesterol, a provitamin in the skin, through exposure to UV from the sun.

Pasture levels of vitamins A and E usually meet all animal requirements for health, with deficiency unlikely to occur. Cases of vitamin A deficiency have been reported among cattle fed with high-concentrate ration indoors, where supplementation would be required. Vitamin E deficiency has similar symptoms to Se deficiency with both being the main biological antioxidants protecting cells from free radical damage. In grazing animals, vitamin E intakes will meet requirements, although differences between pasture species have been noted, for example, red clover has significantly lower levels than grasses (Lee et al., 2009). These differences would more likely manifest themselves in terms of product quality than animal health, as levels of vitamin E in animal products, particularly red meat, are associated with the extension of shelf life. The shelf life of red meat in retail display is driven by colour, unlike white meat which is more related to microbial spoilage. As red meat is temporally oxidised, with exposure to air, myoglobin and oxymyoglobin (red) turn to metmyoglobin (brown). Pasture-fed animals often have muscle tissue with higher levels of PUFA (see the previous section on lipids) which increase the oxidation potential and the formation of free radicals. However, high levels of vitamin E from the diet can counter this oxidation and result in an extended shelf life (Warren et al., 2008). Therefore, pasture which provides a good supply of vitamin E is essential not only for animal health but also product acceptability.

5 Other nutritional factors

Of course, plants are not just proteins, lipids, carbohydrates, minerals and vitamins: there are other compounds which will have a beneficial effect on animal nutrition, such as specific enzymes and active compounds. PPO (mentioned previously) is one such enzyme, associated with improving the protein and lipid metabolism in the rumen. PPO is predominantly associated with the detrimental effect of browning fruit and vegetables. However, interest in PPO-containing forage crops has grown since the browning reaction was associated with reduced N losses *in silo* and the rumen. The reduction in protein breakdown *in silo* of red clover (high PPO forage) increased the quality of protein, improving NUE when fed to ruminants (Lee, 2014). A further benefit of feeding red clover silage is a significant reduction in lipolysis (cleaving of glycerol-based lipid) *in silo* and an increase in the deposition of beneficial C18 PUFA in animal products, which has also been linked to PPO activity. PPO's protection of plant protein and glycerol based-PUFA *in silo* is related to the deactivation of plant proteases and lipases. This deactivation occurs through PPO catalysing the conversion of diphenols to quinones which bind with cellular nucleophiles

such as protein, reforming a protein-bound phenol (PBP). If the protein is an enzyme (e.g. protease or lipase), the complexing denatures the enzyme. However, PPO is inactive in the anaerobic rumen and therefore any subsequent protection of plant protein and glycerol-based PUFA in the rumen must be because of events that occurred to the forage pre-ingestion. Reduced activity of plant proteases and lipases would have a little effect on NUE and glycerol-based PUFA in the rumen due to the greater concentration of rumen microbial proteases and lipases. The mechanism for PPO's protection of plant protein in the rumen is a consequence of complexing plant protein, rather than protease deactivation per se. These complexed proteins reduce protein digestibility in the rumen and subsequently increase the UDP flow to the small intestine. The mechanism for protecting glycerol-based PUFA has yet to be fully elucidated but may be associated with entrapment within PBP, reducing access to microbial lipases or differences in rumen digestion kinetics of the forage and therefore not related to the PPO activity (see review by Lee, 2014).

Relatively low levels of condensed tannins (CT; another phenolic compound) in forage could result in protein being partially protected from the degradation by rumen microbes and improve NUE. CT have also been shown to reduce the incidence of bloat, an abnormal distension of the rumen caused by excessive retention of fermentation gases, which can be found on high-legume-containing diets. However, CT can also be an ANF, reducing digestion in the rumen (see below). Tannins are one example of a large complex group known as plant secondary metabolites (PSM) which also include essential oils, the potential nutritional value of which have been summarised in a review by Acamovic and Brooker (2005). Such compounds have a wide range of biological activities and enormous potential for uses in agriculture, which requires in-depth investigation and evaluation in the context of domesticated livestock production. A group of PSM not covered in the review are the 'green odour compounds', described as the collection of volatile organic compounds (alkenes, jasmonates, hydroperoxides, aldehydes and ketones) released through the action of lipoxygenase activity following cell damage, such as mastication at grazing. These green odour compounds have been implicated in altering lipid metabolism in the rumen reducing biohydrogenation and thus improving the flow of PUFA from the rumen (Huws et al., 2013).

Choline is a vital micronutrient, sometimes referred to as a vitamin, which is essential in a range of cellular functions. Choline and its metabolites are needed for three main physiological purposes: structural integrity and signalling roles for cell membranes, cholinergic neurotransmission (acetylcholine synthesis) and a major source for methyl groups for the formation of methionine from homocysteine and of creatine from guanidoacetic acid. Methyl groups function in the synthesis of purine and pyrimidine (components of DNA). Forage is a rich source of choline through phosphatidylcholine, which makes up a high proportion of plant membrane lipids, especially of the chloroplast (see the previous section on lipids). Low-forage diets may result in low levels of choline, which is rapidly metabolised in the rumen to form trimethylamine. Erdman et al. (1984) reported a significant increase in milk yield and fat percentage when choline was supplemented to a high concentrate:maize diet fed to high-yielding dairy cows.

6 Anti-nutritional factors

Despite the clear nutritional value that pasture delivers to livestock, there are some limiting factors that must be considered which have negative effects on the animal production. The

category ANF includes naturally occurring substances which reduce either nutrient utilisation or feed intake, or cause anti-physiological effects, for instance, by impairing reproduction or reducing immunocompetence (D'Mello, 2000). In pasture species, such compounds include lignin (discussed previously), tannins, nitrates, saponins, phyto-oestrogens, thiaminases and mycotoxins among others. The deleterious effect of these substances varies with factors associated with plant species present, environmental conditions and animal sensitivity.

CTs are the most abundant type of tannins in economically important plant species such as some forage legumes and grains. Concentrations of CT above 50–55 g/kg DM generally reduces voluntary feed intake and digestibility, depresses rates of body and wool growth in grazing ruminants (Min et al., 2003; Piluzza et al., 2014) and decreases *in vitro* DM disappearance and VFA production (Chiquette et al., 1988).

Nitrate in forage is converted to nitrite in the rumen and further metabolised to ammonia. However, when forages have an unusually high concentration of nitrate following N fertilisation and the introduction of grazing animals too soon back to pasture, nitrite accumulates in the rumen and is absorbed into the bloodstream. It then interacts with haemoglobin to form methaemoglobin, which is incapable of transporting or releasing oxygen to the body tissues, a condition known as methaemoglobinaemia. Animal performance will be significantly reduced, and in more severe cases can cause death from asphyxiation, or lack of oxygen (Wright and Davison, 1964).

Hepatogenous photosensitisation disorders are linked with the consumption of plants containing steroidal saponins, such as *Brachiaria decumbens* and various *Panicum* species (D'Mello, 2000). The accretion in the hepatocytes of crystals composed of insoluble Ca^{2+} salts derived from saponins leads to an obstruction of the biliary system. This triggers the accumulation of phylloerythrin, a photosensitising agent produced via the metabolism of chlorophyll in the rumen (see Lee et al., 2010 for a detailed summary of chlorophyll catabolism in the rumen), which circulates in the blood stream causing severe skin blistering, especially in regions more exposed to sunlight, such as the ears (D'Mello, 2000).

The phyto-oestrogens are a diverse group of isoflavonoid compounds found primarily in legumes. Formononetin is the predominant isoflavone found in subterranean clover (*Trifolium subterraneum*) and red clover. Subterranean clover may contain up to 5% DM of oestrogenic isoflavones which varies with cultivar (Adams, 1995). For red clover, the level of phyto-oestrogens is primarily under genetic control. However, environmental factors play a greater role; pasture is most oestrogenic in spring, and oestrogenicity declines after flowering (Adams, 1995). The mechanisms which trigger reproductive dysfunction in animals vary with species. Thus, ewes grazing Lucerne (*Medicago sativa*), with up to 600 ppm of coumestan (its phyto-oestrogenic compound), exhibit a depressed ovulation rate and lambing by 34% and 14.6%, respectively (Smith et al., 1979). However, infertility in cattle is due to anovulation, or/and the development of cystic follicles (Reed, 2016). Nevertheless, the phyto-oestrogenic effects of these legumes depend on the dose and route of exposure, as well as timing since different tissues have species-specific windows of sensitivity to morphological and functional disruptions (Reed, 2016).

As already reported, ruminants can acquire all B vitamins through the symbiotic relationship with their rumen microbial population. However, certain plants such as horsetails (*Equisetum* spp.) and bracken (*Pteridium aquilinum*), more common in upland pasture grazing, contain thiaminases which reduce the uptake of thiamine (vitamin B1) from the rumen, resulting in a neurological condition cerebrocortical necrosis also known as polioencephalomalacia. Although the condition is predominately associated with the

intake of thiaminases, it has also been shown to manifest in animals who have had a rapid dietary change resulting in a ruminal imbalance influencing the microbial ecosystem, such as a rapid switch to high-concentrate diets or movement to lush, highly digestible pasture. Another condition associated with a rapid switch to lush pasture is fog fever or acute bovine pulmonary oedema and emphysema which is associated with the rapid exposure of the rumen to highly digestible protein, especially the amino acid tryptophan. Tryptophan is converted by rumen microbes to 3-methylindole which is absorbed across the rumen and circulated around the body where it is toxic to the primary cells that line the interior surface of the lungs.

Mycotoxins are fungal toxins produced predominately by *Aspergillus* spp., *Fusarium* spp. *and Penicillium* spp. associated with cereals, although maize silage is also a major culprit in dairy cow rations (Cogan et al., 2016) and significant mycotoxins were also found in grass silage in a recent survey of big bales and clamps in Ireland (McElhinney et al., 2016). Mycotoxins can also cause issues at pasture. Ryegrass staggers, a neurological condition, is caused through the ryegrass fungal endophyte *Acremonium lolii* which produces lolitrem B which acts as a potent large-conductance Ca-activated K-(BK) channel inhibitor causing muscle spasms. The amounts of fungal hyphae and lolitrem B in infected plants increase to toxic levels as the temperature rises in late spring and decrease again to safe levels in the cooler seasons. Similarly, ergot alkaloids are toxins produced by *Claviceps purpurea*, which are common pathogens of various grass species. Although predominately associated with grain crops, they have been found within grass seed heads in mature swards. These alkaloids affect the blood flow to the extremities and in some extreme cases result in the loss of ears and hooves.

7 Future trends and conclusion

Grazed pasture is the single most important forage feed for ruminants due to its low unit cost and widespread global availability. However, CAST (1999) estimated that between 1993 and 2020, the growth in cereal grain used as livestock feed would be 1.4% per annum, comprising annual growth rates of 2.7% in developing countries and 0.7% in developed countries. With global livestock numbers expected to exceed 35 billion chickens, 2.5 billion cattle (all bovines), 2.5 billion sheep and goats, one billion pigs and 25 million camels (Thornton, 2010), by 2050, the quantity of arable crops given to livestock might exceed that used by humans (Bailey et al., 2014). The challenge for the future development of ruminant systems is to ensure that human-edible feeds, or preferably by-products if available locally, are used solely to strategically complement pastures to optimise performance where needed with the performance driven by high-quality forage grown on farm (Wilkinson and Lee, 2017). To achieve this, pastures need to be considered for their nutritional value (energy, protein, minerals and vitamins), and grazing and plant and animal breeding strategies developed accordingly to optimise performance and quality from pasture.

Research is needed to determine how best grazing systems should be utilised in order to deliver sustainable ruminant livestock systems as a part of global food security. The North Wyke Farm Platform, a UK National Capability (www.nwfp.rothamsted.ac.uk), provides a means to research, understand and deliver sustainable grazing livestock systems through developing a detailed understanding of sustainability metrics and trade-offs (e.g. productivity, nutritional quality, health and welfare, biodiversity and emissions). The aim is

to deliver optimised production of high value animal products from land not suitable for crop production at the same time as harnessing and protecting other ecosystem services and minimising environmental pollution (Orr et al. 2016; Takahashi et al., 2017).

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